

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
6 December 2001 (06.12.2001)

PCT

(10) International Publication Number
WO 01/92570 A2

- (51) International Patent Classification⁷: C12Q 1/68 (81) Designated States (*national*): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.
- (21) International Application Number: PCT/GB01/02338
- (22) International Filing Date: 25 May 2001 (25.05.2001)
- (25) Filing Language: English
- (26) Publication Language: English
- (30) Priority Data:
0012860.3 27 May 2000 (27.05.2000) GB
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- (84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/92570 A2

(54) Title: METHOD TO SCREEN FOR IMPROVED MEAT CHARACTERISTICS IN PIGS

(57) Abstract: There is provided an assay to identify pigs having a genetic predisposition to musculature with improved meat quality characteristics. In the assay certain genetic markers which correlate to the meat quality traits of interest are used to determine the allelic variant(s) in the DNA sample under test. Preferred markers are: i) SW413, SW1482, SW439, S0005, SW904 or regions of chromosome 5 spanning therebetween; or ii) SWR68, S0024, SW827, SW727, SW539, or regions of chromosome 9 spanning therebetween; or iii) SW2093, SW2116 or regions of chromosome 9 spanning therebetween. From the genotypic data so generated pigs of the preferred genotype can be selected for slaughter or for use in breeding programs. A kit for conducting the assay is also described.

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1 METHOD TO SCREEN FOR IMPROVED MEAT CHARACTERISTICS
2 IN PIGS
3

4 The present invention relates to pigs having
5 musculature with improved meat quality, and ways to
6 identify them, including muscle fibre
7 characteristics and genetic markers. In particular,
8 the present invention provides an assay to screen
9 pigs for improved meat quality characteristics such
10 as tenderness, shear force and muscle fibre
11 characteristics.
12

13 In the United Kingdom, elsewhere in Europe and
14 increasingly throughout the world, pig producers
15 are selecting breeds to use on their farms that are
16 efficient producers of lean meat of high quality
17 and thus provide the farmer with the maximum
18 possible economic return.
19

20 'White' breeds of pig, like the 'Large White' and
21 'Landrace' especially those produced by pig
22 breeding companies in the United Kingdom are

1 characterised by having a good growth rate and
2 producing carcasses with a low subcutaneous and
3 intermuscular fat level and thus a high lean
4 content. These characteristics also lead to animals
5 with a high feed conversion efficiency.

6 Considerable progress in improving the lean meat
7 content of these breeds of pig has been made in
8 recent years in the United Kingdom.

9
10 There are reasons to believe that this long-term
11 selection for lean content may have had the
12 consequence of coincidentally selecting for pigs
13 with a biological predisposition to poor meat
14 quality. In particular, the lean meat may be
15 increasingly predisposed to a problem known as Pale
16 Soft Exudative meat (PSE), and may have eating
17 quality problems such as toughness and dryness.

18
19 Another important world breed of pig is the
20 'Duroc'. This is a North American breed of meat
21 pig, red in colour and originating between 1822 and
22 1877 from 'Old Duroc' of New York and 'Jersey Red'
23 of New Jersey. A breed society was formed in 1833
24 (Mason 1988). The 'Duroc' remains very popular in
25 the United States and many were imported into
26 Europe during the twentieth century.

27
28 Within Europe, especially the United Kingdom, the
29 'Duroc' is characterised as being of reasonable
30 growth rate, but fatter and less efficient with
31 regard to meat production than 'Large White' and
32 'Landrace'. However, it has been shown a number of

1 times to have meat of superior quality, especially
2 colour and tenderness, than the "White" breeds (as
3 defined above).

4
5 In Canada, Denmark, France and New Zealand, pigs
6 produced from "White" hybrid mothers and 'Duroc'
7 sires
8 have produced pigs with a tenderness advantage
9 ranging from 10 to 17% over similar but 'White'
10 sired pigs (Martel et al 1988; Barton-Gade 1989;
11 Gandemer and Legault 1990 and Purchas et al 1990).

12
13 The interest in the 'Duroc' breed in the United
14 Kingdom prompted the Meat and Livestock Commission
15 to undertake what is probably the most
16 comprehensive evaluation of the breed ever done.
17 Conventional 'White' British commercial pigs
18 ('Large White' sires crossed to 'Large White' cross
19 'Landrace' dams) containing zero percent 'Duroc'
20 genes were compared with pigs containing 25, 50 or
21 75% 'Duroc' genes produced by various crosses (MLC
22 1992). Some results for 0% and 50% 'Duroc' pigs
23 (ie. 100% and 50% "White" pigs) are presented in
24 Table 1 and illustrate the relative merits of the
25 two pig types.

26

1 Table 1

	DUROC CONTENT	
	0%	50%
Daily live weight gain (g)	806	803
Feed conversion ratio	2.70	2.83
Lean tissue feed conversion ratio	6.19	6.81
P ₂ fat depth (mm)	9.3	10.9
Lean %	58.8	56.6
PSE carcasses (%)	8.3	1.6
Deep seated hair (% carcasses)	1.1	17.6
Tenderness score*	4.96	5.32
Pork flavour*		
In lean	3.88	3.96
In fat	3.87	4.06
Pork odour in fat*	3.58	3.73

2

3

4 * sensory scores are on a 1-8 scale where higher
5 scores indicate more tender, juicy etc. All
6 results are for pigs fed ad-libitum but
7 restrictedly fed pigs show similar results (MLC
8 1992).

9

10 Thus it can be seen that 'Duroc' cross pigs have
11 good quality meat in comparison to 'White' pigs but
12 this is obtained at the expense of being less

1 efficient, fatter and having other carcase quality
2 problems.

3

4 The difference between 'White' and 'Duroc' with
5 regard to tenderness illustrates the existence of a
6 genetic component for meat quality traits, that may
7 equally exist between other breeds or within breeds
8 or crosses. It is not a proof that the 'Duroc'
9 always has better meat quality than 'White', the
10 reverse may also be true on occasions.

11

12 Tenderness is a particularly important trait
13 because, as described by Warkup et al (1995),
14 previous experience of the product plays a major
15 role in the consumer's decision to buy it again.
16 Unlike attributes like the animal's welfare,
17 residues and food hygiene (unless consumption
18 results in illness), sensory attributes are
19 actually experienced by the consumers. Studies
20 quoted by Warkup et al (1995) indicate that
21 tenderness is the most important attribute of meat.

22

23 The sensation of tenderness by a consumer can be
24 assessed by a trained taste panel. Trained panels
25 operating under strictly controlled conditions are
26 able to detect smaller differences in tenderness
27 and other meat quality traits than the consumer at
28 large. Example 1 includes a description of a
29 trained taste panel operated to assess meat quality
30 attributes.

31

1 Tenderness of meat can also be measured
2 instrumentally, and is then defined as the shear
3 force. The force required to cut through a piece of
4 meat is measured and can be expressed as the force
5 at first yield, total work and maximum force or
6 related traits. Example 1 includes a description
7 of exemplary measurement of shear force traits.

8
9 Correlations between shear force and taste panel
10 scores for tenderness (with low scores for tender
11 meat and high scores for tough meat) vary from 0.27,
12 to 0.78 (Stumpe 1989).

13
14 To date there is no clear explanation of what
15 causes the meat quality differences between White
16 breeds and Duroc. There is a widely held belief
17 that the level of fat in the muscle (intramuscular)
18 fat may be important (Bejerholm 1984) but there are
19 contradictory views about the role of fatness and
20 the 'Duroc' clearly differs from 'White' pigs in
21 more respects than just fatness.

22
23 One of the observations made in our own earlier
24 studies (MLC 1992) was that pigs containing 'Duroc'
25 genes have a higher level of haem pigment. This
26 observation and the higher levels of intramuscular
27 fat are an indication of a higher oxidative
28 capacity in the muscle.

29
30 Muscle (and hence meat) is made up of a variety of
31 different muscle fibre cell types, which can be
32 classified according to their contractile and

1 metabolic nature. The two major classes of fibre
2 type identified on the basis of their contractile
3 nature (fast twitch and slow twitch) are subdivided
4 into a number of subtypes based on their metabolic
5 nature. Thus, according to one method of
6 classification (see Peter et al 1972) muscle is
7 shown to comprise slow-twitch oxidative (SO),
8 fast-twitch glycolytic (FG), fast-twitch
9 oxidative/glycolytic (FOG) and fast-twitch
10 oxidative muscle fibre types. The proportions of
11 the fibre types vary between muscles.

12
13 These fibre types are common to most muscles from
14 most meat animals and typically show a random
15 distribution throughout the tissue. However, in the
16 pig the SO fibres are arranged with clusters or
17 groups and are surrounded by fast twitch fibre
18 types (Szentkuti and Cassens 1978). This
19 association of muscle cells of similar metabolic
20 types was described as forming "metabolic" clusters
21 (Handel and Stickland 1987). The number of SO
22 clusters is believed to be proportional to the
23 number of primary fibres formed during myogenesis,
24 the number of primary fibres being fixed in the pig
25 foetus by 70 days gestation.

26
27 There is evidence of differences in the proportions
28 of these different fibres among pig breeds (Iwamoto
29 et al 1983; Ruusunen 1993). Differences in
30 proportion of different fibre types have also been
31 shown to occur among different pig breeds when
32 fibre proportion is analysed for bundles of mixed

1 fibre types (Skorjanc et al 1994). There has also
2 been a tendency for breed crosses including 'Duroc'
3 to have more SO and more FOG fibres (Uhrin et al
4 1986). This latter observation is entirely
5 consistent with the proposed higher oxidative
6 capacity as indicated by higher haem content.

7
8 The clearest breed difference in SO frequency was
9 that seen by (Ruusunen 1993). These workers
10 examined the fibre type composition of the
11 *Longissimus Dorsi* of 38 pure 'Hampshire' (H), 52
12 'Finnish Landrace' (L) or 'Yorkshire' (Y) sires
13 cross onto (L x Y females), and 52 H sires crossed
14 onto (L x Y females) pigs. SO frequency was 15.3%,
15 11.5% and 11.6% respectively. The H had
16 significantly more SO fibres than either cross. The
17 fibre composition of the H cross animals more
18 closely resembled the composition of the animals
19 which did not contain H than the pure H animals.
20 This confirms that breed differences for meat
21 quality characteristics are not limited to
22 comparisons including 'Duroc'.

23
24 Results from recent studies of porcine longissimus
25 muscle, presented in WO-A-98/15837 show:

- 26
27 1. That the percentage frequency of SO fibres and
28 the proportional area of SO fibres per unit
29 muscle is increased in the Duroc pig relative
30 to the "White" pig;

31

- 1 2. That the number of SO fibres per cluster is
2 increased in the Duroc pig relative to the
3 "White" pig;
4
 - 5 3. That m calpain is preferentially localised in
6 the SO fibres of pigs. Therefore pigs with
7 more SO fibres (eg Duroc) have more m calpain
8 in the muscle as a whole. Thus the amount of
9 m calpain is increased per unit muscle in the
10 Duroc pig relative to the "White" pig;
11
 - 12 4. That the amount of μ calpain per fibre is
13 increased in the Duroc pig relative to the
14 "White" pig;
15
- 16 It is well documented that post mortem storage of
17 animal carcasses at below ambient temperature, but
18 above freezing, results in an improvement in meat
19 tenderness. This increase in tenderness is due to
20 the enzymatic breakdown of myofibrillar proteins
21 and there is evidence that calpains are responsible
22 for 90% of the tenderisation that occurs during
23 post mortem storage (Taylor et al 1994). Calpains
24 are intracellular, calcium activated/dependent
25 thiol proteases present to some extent in most body
26 tissues. However, their exact role in normal
27 physiological conditions is still undefined.
28 Several isoforms of calpain are known to occur in
29 various body tissues of birds and animals. Two
30 isoenzymes, μ calpain and m calpain, with different
31 calcium requirements were originally isolated

1 (Huston and Krebs 1968, Mellgren 1980). More
2 recently tissue specific calpains have been
3 isolated from skeletal muscle and stomach
4 (Sorimachi et al 1989, Sorimachi et al 1993). It
5 is the actions of μ calpain and m calpain that are
6 thought to be involved in post mortem tenderisation
7 of meat. In animal carcasses μ calpain is most
8 active during the first 15 hours post slaughter
9 whereafter its activity declines rapidly whilst the
10 activity of m calpain is much more persistent. The
11 activity of both μ and m isoforms of calpain is
12 regulated by a natural inhibitor, calpastatin,
13 which is also ubiquitously distributed in all body
14 tissues.

15
16 Studies presented in WO-A-98/15837 have shown that
17 m calpain is concentrated in the SO fibres of pig
18 muscle. As Duroc meat has a greater proportion of
19 SO fibres compared to meat from other breeds the
20 corresponding increase in m calpain levels could
21 account for the tenderness of Duroc meat.

22
23 It was also found that there is an overall
24 increased amount of μ calpain per fibre in the
25 muscles of Duroc pigs. An increased concentration
26 of μ calpain per fibre could also explain the
27 increased tenderness of Duroc meat.

28
29 Selection of animals with a genetic predisposition
30 to better meat quality would be an attractive and
31 cost-effective method to improve meat quality. The

1 identification of animals of the desired genotype
2 (genetic make up) requires some understanding of
3 the nature of genetic variation and methods to
4 detect it.

5

6 An animal's phenotype is the result of complex
7 actions of the genes inherited from its parents and
8 environmental factors. Most traits of agricultural
9 importance in animal production are influenced by
10 variation at several or many different genes.

11 Usually individual genes do not have a large enough
12 effect on their own to produce observable
13 qualitative differences between individuals. More
14 commonly, variation in several or many genes
15 combines to produce continuous or quantitative
16 variation between animals in traits such as growth
17 rate and fatness.

18 Genome mapping can be used to identify the location
19 of genes that influence variation in quantitative
20 traits. The loci affecting quantitative traits are
21 termed quantitative trait loci or QTLs.

22 The tools used to follow the inheritance in
23 different chromosomal regions are genetic markers
24 and these can be selected from the genome map to
25 ensure coverage of the entire genome.

26

27 Maps showing distances between ordered loci can be
28 built using recombination frequencies between pairs
29 of loci or between multiple groups of loci.

30

31 For example, linkage maps of the porcine genome now
32 contain substantial amounts of information and

1 their status is constantly changing. Published
2 linkage maps and linkage data are stored in the
3 genome databases, for the pig this is PiGBASE /
4 ARKdb-pig: URL = <http://www.thearkdb.org>.

5
6 The basic principle of showing a gene or a region
7 of the genome is associated with variation is
8 illustrated in Figure 1 for pigs. It consists of
9 identifying a genetic marker and showing that its
10 inheritance in a suitable pedigree is associated
11 with variation in performance.

12
13 In a population such as that derived from the cross
14 between two lines illustrated in Figure 1, there
15 may be an overall association between a particular
16 marker allele and a particular allele at a
17 quantitative trait locus (QTL). Linkage
18 disequilibrium between a QTL and a marker leads to
19 an overall association between the marker allele
20 and the quantitative trait. In a random mating
21 population, recombination over a number of
22 generations will lead to the gradual decay in
23 linkage disequilibrium between loci, with the rate
24 of decay related to the distance between the loci.

25
26 Genome studies often analyse several or many
27 different markers when looking for an effect on the
28 phenotype. Thus, a number of effects may be
29 significant by chance if the standard 5%
30 significance level is used. Hence, it is
31 recommended practise to use a more stringent
32 significance level such that the overall chance of

1 finding a significant result amongst all the
2 markers tested is no more than 5% (see Lander and
3 Kruglyak (1995) for a more detailed discussion of
4 these points). This means that nominal
5 significance levels at 0.01-0.001% or higher may be
6 used in some studies. This in turn increases the
7 sample size required for results to be significant
8 at this level.

9
10 In genome scans for pigs where 19 chromosomes are
11 tested and many positions within chromosomes, use
12 of the nominal threshold is likely to lead to a
13 number of false positive results reaching this
14 significance threshold. Hence, QTL results are
15 usually judged against a genome wide significance
16 threshold (probability of a false positive for a
17 single trait <0.05 in the entire genome, equivalent
18 to an F value >9.0 for the pig genome) or the less
19 stringent genome wide suggestive significance
20 threshold (expect one false positive per trait in a
21 whole genome scan, equivalent to $F>5.0$
22 approximately in the pig genome). See table below
23 for further clarification:

24

25 Expected number of false positives in scan of:

Threshold	F value	Whole genome	One chromosome	Single Point
Nominal	>3.0	ca. 5	ca. 0.25	0.05
Suggestive	>5.0	1.0	0.05	0.01
Significant	>9.0	0.05	<0.001	<0.0001

26

27

1 The full power of the map and markers is employed
2 in performing a genome scan for loci affecting
3 traits of interest. The strength of this approach
4 is that it has the potential to detect any loci
5 with a large effect on a studied trait, whether or
6 not their existence is known in advance. To
7 implement this approach, markers which are spaced
8 at intervals through the genome and which are
9 polymorphic in the population being studied are
10 selected from the map. The phenomenon of genetic
11 linkage means that each marker can be used to
12 follow the inheritance of a section of linked
13 chromosome. Around 100-150 evenly spaced markers
14 are needed to cover the whole genome and follow the
15 inheritance of all sections. Thus maps of highly
16 polymorphic markers are very valuable for this
17 approach, as they allow selection of markers that
18 provide this coverage and that are informative in
19 the population of interest.

20

21 Thus the genome scan can both localise known genes
22 of major effect and identify loci that were not
23 known *a priori*. A significant amount of work is
24 required to type sufficient animals for markers
25 covering the entire genome. However, it is
26 possible to design an experiment such that there is
27 a high probability of detecting a gene of a defined
28 effect on the phenotype wherever it is in the
29 genome. More details on genome scans can be
30 accessed in research publications, review articles
31 and textbooks.

32

1 We have conducted such a genome scan for QTL
2 contributing to variation in meat quality and its
3 component traits; including muscle fibre
4 characteristics.

5

6 The present invention is concerned with the use of
7 genetic markers to identify animals with superior
8 genes for meat quality traits.

9

10 The invention is founded upon the following novel
11 observations:

12

13 1. Pig genetic markers SW413, SW1482, SW439, S0005
14 and SW904 or regions of chromosome 5 spanning
15 therebetween are associated with shear force,
16 muscle fibre characteristics and eating quality
17 and related meat quality traits;

18

19 2. Pig genetic markers SWR68, S0024, SW827, SW727
20 and SW539 or regions of chromosome 9 spanning
21 therebetween are associated with muscle fibre
22 characteristics, shear force, tenderness and
23 related meat quality traits;

24

25 3. Pig genetic markers SW2093 and SW2116 or regions
26 of chromosome 9 spanning therebetween are
27 associated with muscle fibre characteristics and
28 related meat quality traits;

29

30 Note that the observed genetic effects are
31 different from those found by Soumillion et al 1997
32 who established an association between meat fibres

1 and the Myogenin gene, located at the middle of pig
2 chromosome 9.

3

4 The specific markers referred to above detailed in
5 the website <http://www.thearkdb.org> and brief
6 details of these markers are also set out in
7 Example 1.

8

9 Experimental details, including primer sequences
10 for many of the genetic markers, can also be found
11 on the USDA Meat Animal Research Centre, WWW site
12 at <http://sol.marc.usda.gov>.

13

14 The present invention provides an assay to identify
15 pigs with a genetic predisposition for improved
16 meat quality, wherein said assay comprises:

- 17 a) obtaining a DNA sample from a test pig;
- 18 b) analysing the sample to determine the allelic
19 variant(s) present at a genetic marker,
20 wherein said marker is selected from:
 - 21 i) SW413, SW1482, SW439, S0005, SW904 or
22 regions of chromosome 5 spanning
23 therebetween; or
 - 24 ii) SWR68, S0024, SW827, SW727, SW539, or
25 regions of chromosome 9 spanning
26 therebetween; or
 - 27 iii) SW2093, SW2116 or regions of chromosome 9
28 spanning therebetween; and
- 29 c) using the genotypic data from said marker(s) to
30 select for pigs of the preferred genotype.

31

1 By "improved meat quality" or "high meat quality"
2 we refer to animals which yield meat exhibiting
3 desirable traits of tenderness and shear force.
4

5 For clarity it should be understood that the assays
6 referred to herein may be conducted on individual
7 animals or, for reasons of economy, may be
8 conducted on pooled genetic samples for a group of
9 animals.
10

11 In a yet further aspect, the present invention
12 provides a method of identifying pigs which have a
13 genetic disposition for improved meat quality, said
14 method comprising:
15

- 16 a) obtaining a DNA sample from said pig;
- 17 b) assaying said DNA sample for a sequence
18 identical with or complementary to the genetic
19 markers identified above.
20

21 The animals identified by the assays referred to
22 herein may be slaughtered to provide high quality
23 meat and/or may also be selected for breeding
24 programs.
25

26 Accordingly the present invention also provides a
27 method of selecting pigs for use in breeding
28 programs, said method comprising obtaining a DNA
29 sample from a test pig and analysing said sample to
30 determine the allelic variant(s) present at a
31 genetic marker as described above, and using the

1 genotypic data from said marker to select for pigs
2 having the required genotype.

3
4 Although the study looked at the particular markers
5 identified above, it is known to those skilled in
6 the art that other genetic markers from within the
7 QTL or the neighbouring portions of pig chromosome
8 5 or 9, or their homologues in other mammals (as
9 appropriate) may be used instead, provided of
10 course that the marker(s) selected are found to map
11 within or close to the QTL for meat quality traits.

12
13 Thus, the present invention provides a method to
14 identify pigs with a genetic predisposition for
15 improved meat quality, wherein said method
16 comprises:

- 17 a) obtaining DNA samples from a population of
18 pigs;
- 19 b) genotyping at least a sample of said
20 population for pre-determined markers that map
21 within or close to the QTL for meat quality
22 traits defined herein (preferably on
23 chromosomes 5 and 9, for example the specific
24 markers referred to above or other markers
25 located on either of chromosomes 5 and 9 where
26 a high F ratio is indicated in any of Figs. 2
27 to 5;
- 28 c) measuring meat quality traits for at least a
29 sample of said population;
- 30 d) correlating the presence of allelic variants
31 of said markers with said meat quality traits;
- 32 e) obtaining a DNA sample from a test pig;

- 1 f) analysing the sample to determine the allelic
2 variant(s) present at a said selected genetic
3 marker; and
4 g) using said marker results to select for pigs
5 of the preferred genotype.
6

7 Steps a) and d) of the method described above are
8 concerned with identifying markers which map within
9 or close to the QTL for meat quality traits or with
10 confirmation that the particular markers referred
11 to are also relevant for the test population.
12

13 Preferably for pigs the markers are derived from
14 SW413, SW1482, SW439, S0005, SW904, SWR68, S0024,
15 SW827, SW727, SW539, SW2093 or SW2116. Other
16 markers that map within or close to the QTL
17 described herein can also be used. Particular
18 mention may be made of any marker located on
19 chromosome 5 in respect of shear force, or between
20 or close to SW1482 and SW904 on chromosome 5 in
21 respect of fibre traits, or between or close to
22 SWR68 and SW2093 on chromosome 9 or between or close
23 to SW2093 and SW2116 on chromosome 9. Preferably for
24 other species, markers are derived from regions of
25 the genome that are known to be homologous to the
26 said regions on pig chromosome 5 and 9.
27

28 As can be seen in Figs. 2 to 5 certain regions of
29 chromosomes 5 and 9 correlate to high F ratios for
30 specific traits connected to meat quality and
31 markers in these regions may be of particular
32 interest.

1
2 Optionally, a selection of markers that each allow
3 the allelic variation at different QTL associated
4 with meat quality to be predicted may be used in
5 combination to achieve a more accurate prediction
6 of meat quality predisposition. The present
7 invention thus provides a kit to identify a pig
8 having a genetic disposition for high meat quality
9 said kit comprising at least three such genetic
10 markers, preferably selected from the specific
11 markers recited above, having the ability to
12 identify specific allelic variant(s) at three
13 separate QTL indicative of meat quality.

14
15 The animals shown to have marker genotypes or
16 predicted QTL genotypes indicative of an improved
17 meat quality predisposition, or the close relatives
18 of such animals, can be used as breeding stock or
19 for meat production.

20
21 In a further aspect the present invention provides
22 a method of determining the genetic predisposition
23 of a pig to yield meat of improved meat quality,
24 said method comprising detecting genes located
25 between the following pairs of markers:

26 i) SW413 and SW904 on chromosome 5;
27 ii) SWR68 and SW539 on chromosome 9; and
28 iii) SW2093 and SW2116 on chromosome 9;
29 wherein said genes are characterised by having
30 allelic variant(s) which can influence meat quality,
31 or its component traits, or which are associated

1 with variation in meat quality or its component
2 traits.
3
4 Although the genetic markers used in this study are
5 microsatellites the assay is not limited to the use
6 of any particular technology or type of genetic
7 marker. Any method for detecting DNA variation at
8 specific chromosomal locations can be used to
9 develop genetic markers that could be used for
10 monitoring the inheritance of particular
11 chromosomal segments or loci. It is clear to those
12 skilled in the art that genetic markers, which map
13 close to or within the QTL for muscle
14 characteristics/meat quality traits defined herein,
15 could be used in the assay for predicting on
16 individual's predisposition for meat quality traits
17 independent of the technology used to develop or
18 genotype the marker. Thus, the assay is not
19 limited to any particular type of genetic marker or
20 genotyping technology, current or as yet
21 undeveloped. Other genetic marker types and
22 technologies include, but are not limited to,
23 restriction fragment length polymorphisms (RFLPs),
24 single strand conformational polymorphisms (SSCP),
25 double strand conformational polymorphisms, single
26 nucleotide polymorphisms (SNPs), AFLP™ (amplified
27 fragment length polymorphisms), DNA chips, variable
28 number of tandem repeats (VNTRs, minisatellites),
29 random amplified polymorphic DNA (RAPDs),
30 heteroduplex analyses, and allele-specific
31 oligonucleotides (ASOs). Some DNA variation can be
32 detected by assaying the variation in RNA

1 transcripts or proteins. Thus, genetic marker
2 technology for the purposes of the assay is not
3 limited to direct measures of DNA variation.

4
5 Examples of markers that map to the muscle
6 characteristics and meat quality QTL on pig
7 chromosomes 5 (SSC5) and 9 (SSC9) include, but are
8 not limited to, (marker type and chromosome are
9 shown in parentheses) ACO2 (SSCP, SSC5); DAGK1,
10 IGF1, IFNG (microsatellites, SSC5); MUC (RFLP,
11 SSC5); PLP1 (protein variants, SSC5); EAE, EAK
12 (erythrocyte antigen variants, SSC9); PPP2R1A, TYR,
13 DLD (RFLPs, SSC9); MYOG (PCR-RFLP, SSC9); APOA1
14 (microsatellite, SSC9). Details of genetic marker
15 technology can be accessed in primary research
16 publications, review articles, textbooks and
17 laboratory manuals.

18
19 Genes that map to the QTL regions identified on
20 chromosomes 5 or 9 can be considered candidates for
21 the genes determining the observed effects on meat
22 quality traits. The basis of the candidature of
23 these genes is their chromosomal locations. Hence,
24 these genes are 'positional' candidate genes.
25 Genes whose map location in pigs is currently
26 unknown but which can be predicted to map to the
27 QTL regions on chromosome 5 or 9 from knowledge of
28 the map location of homologous genes in humans,
29 mice and other species can be considered as
30 'comparative positional' candidates for the genes
31 determining the observed meat quality traits.

32

1 Positional and comparative positional candidate
2 genes determining functions that may contribute to
3 the observed meat quality traits include, but are
4 not limited to, the genes encoding: myogenic factor
5 5 (MYF5); myogenic factor 6 (MYF6); collagen type
6 II, alpha 1 (COL2A1); insulin-like growth factor 1
7 (IGF1); myosin phosphatase, target subunit 1
8 (MYPT1); myosin-binding protein C, slow-type
9 (MYPC1); Wnt inhibitory factor 1 (WIF1); growth
10 differentiation factor 11 (GDF11) and myogenin
11 (MYOG). To those skilled in the art the isolation
12 of the pig homologues of such candidate genes and
13 the subsequent search for causal genetic variation
14 in the candidate gene(s) is straightforward.

15
16 In the assay of the present invention, the genomic
17 DNA will be detected from a sample of tissue
18 donated from the pig, but the exact tissue forming
19 the sample is not critical as long as it contains
20 genomic DNA. Examples include (but are not limited
21 to) body fluids such as blood, semen (sperm),
22 ascites and urine; tissue and cells such as liver
23 tissue, muscle, skin, hair follicles, ear, tail,
24 fat and testicular tissue. The genomic DNA to be
25 analysed can be prepared by extracting and
26 purifying the DNA from such samples according to
27 standard laboratory procedures.

28
29 The method may be conducted *in vitro* or *in vivo*
30 using a sample from a living animal or post mortem
31 following the death of the animal being tested. If
32 the assay is conducted post mortem, the information

1 obtained may be also of use for the siblings,
2 parents or other close relatives of the animal.
3

4 The QTL for meat quality traits disclosed herein
5 will allow the isolation and characterisation of
6 the trait-genes themselves in pigs, since the
7 positioning of the QTL enables a search for linkage
8 to the genes responsible for the trait. Once these
9 trait genes are located the option to manipulate
10 the trait genes by transgenesis or to develop a
11 further assay arises and forms part of the present
12 invention.
13

14 Various genes and/or controlling sequences may be
15 involved, especially the genes controlling the
16 calpain/calpastatin system.
17

18 The invention will now be described with reference
19 to the following, non limiting, examples and
20 figures in which:
21

22 Figure 1 depicts a three-generation pig pedigree
23 produced by crossing divergent purebred lines of
24 pigs to produce F_1 and F_2 generations. We focus on
25 one small part of a single chromosome that carries
26 a genetic marker with alternative alleles 1 and 2.
27 The animals can be genotyped for this marker and
28 the inheritance of alternative alleles can be
29 followed through the pedigree. In the F_2 animals,
30 both the marker and genes controlling the size
31 differences between the breeds segregate. The
32 marker acts as a signpost to show from which breed

1 linked sections of chromosome are inherited. In
2 this example the size of F_2 animals is associated
3 with the marker genotype (animals with the 11
4 genotype are large, those with 22 are small).
5 Hence a gene or genes for size is found in the
6 region of chromosome inherited with the marker.

7
8 Figures 2 and 4 are graphs plotting the F value
9 against position (cM) on pig chromosome 5 for
10 different meat quality related traits.

11
12 Figure 3 and 5 are graphs plotting the F value
13 against position (cM) on pig chromosome 9 for
14 different meat quality related traits.

15
16 Example 1

17 QTL analysis

18
19 QTL mapping pedigrees were established in the form
20 of three-generation families in which grandparents
21 from genetically divergent breeds were crossed to
22 produce the parental (F_1) generation which were
23 subsequently intercrossed. The founder
24 grandparental breeds were the Duroc and the
25 European Large White (Yorkshire). About 120 F_2
26 animals were produced in these Large White/Duroc
27 pedigrees.

28
29 Blood or tissue samples were taken from most
30 grandparental, F_1 parental and F_2 pigs and these
31 were used to prepare DNA.

32

1 Taste panel, shear force and fibre traits

2

3 The phenotype markers were:

4 i) taste panel assessment of tenderness;

5 ii) taste panel assessment of overall acceptability;

6 iii) taste panel assessment of juiciness, pork flavour,
7 abnormal flavour and boar flavour;

8 iv) shear force measurements as force at first yield,
9 total work and maximum force;

10 v) muscle fibre characteristics traits as described
11 below.

12

13 Tenderness, overall acceptability and the other taste
14 traits (i to iii) were measured by the trained taste
15 panel at the Meat and Livestock Commission. Two samples
16 of meat for each animal were assessed in separate
17 sessions by a trained sensory panel. There was a total
18 of 365 sessions. At each panel session, meat samples
19 from eight animals were analysed. Each of six panellists
20 at that session was then given a separate sample of loin
21 chop of each of the eight animals. Each panellist gave
22 each animal a score for five attributes, on a scale of
23 1-24 (the higher the better) by marking a prepared form.
24 The sample was assessed by mouth for juiciness,
25 tenderness, pork flavour, abnormal flavour and boar
26 flavour. Finally, a score was given for overall
27 acceptability.

28

29 Each session and panellist involved in the trial had a
30 unique number. The scores awarded by the panellists were
31 analysed using the restricted maximum likelihood in a

1 model fitting session number, panellist and individual
2 animal number. Fitted values for each attribute for each
3 individual were saved from these analyses and stored on
4 a database for use in the QTL analyses.

5

6 For shear force measures (iv) the following
7 protocol was used:

- 8 1) A 120 mm section of forequarter loin was
9 removed anterior to the last rib.
- 10 2) After the removal from the carcass, joints
11 were de-boned and de-rinded, labelled with the
12 appropriate control number and vacuum-packed.
- 13 3) Samples were aged for seven days
- 14 4) In order to ensure uniform rapid freezing,
15 samples were first placed in a blast-freezer
16 before being transferred to the main cold
17 store for storage at -30°C .
- 18 5) On removal from the cold store, samples were
19 placed in the chiller at $+3^{\circ}\text{C}$ for a period of
20 72 hours. Joints were placed on racks,
21 avoiding overlap in order to facilitate
22 consistency of thaw.
- 23 6) At 72 hours, the internal temperature of each
24 joint was checked and only when all samples
25 had internal temperatures of between 2 and 5°C
26 would cooking commence. After reaching the
27 required temperature, each sample was re-
28 vacuum packed and immediately taken to the
29 Sensory Laboratory for cooking to commence.
- 30 7) Samples were placed in the water bath when the
31 water temperature had reached 80°C . Each sample
32 was cooked within its individual vacuum pack.

- 1 One sample was used to monitor internal
2 temperatures. This sample was cooked until the
3 internal temperature reached 80°C, all samples
4 were then cooked for a further 10 minutes.
- 5 8) After completion of cooking, samples were
6 transferred to an iced water bath for one
7 hour. Water was replaced every 15 minutes.
- 8 9) After the one hour period, all samples were
9 taken to the cutting room chiller and stored
10 overnight at +3°C. They were laid on racks in
11 order to ensure good air circulation.
- 12 10) The following day, ten replicate samples, each
13 measuring 10 mm x 10 mm x 30 mm were removed
14 from each sample, cutting each replicate along
15 the direction of the fibres.
- 16 11) Replicates that had obvious tissue defects or
17 did otherwise not represent a sample were
18 discarded. If insufficient meat was available
19 to replace these samples, then a lesser number
20 than 10 was measured. Samples and replicates
21 were kept covered and refrigerated between 2 °
22 C and 5°C until they were sheared.
- 23 12) The instrument used was a TA.XT2i Texture
24 Analyser (Stable Micro Systems, England).
- 25 13) A Volodkevich (Stable Micro Systems, England)
26 bite jaw was fitted.
- 27 14) The jaw was calibrated at 1.7 mm/s and
28 travelled 8 mm into the sample.
- 29 15) The following were recorded on each replica:-
30 - Force at first yield
31 - Total work
32 - Maximum force

1

2 Fibre typing fibre traits (v) were determined as
3 follows:

4

5 Pigs were slaughtered when the mean litter live
6 weight reached 90kg.

7 Loin samples were removed for histochemical and DNA
8 analysis 48 hours after slaughter.

9

10 The histochemical analysis of the muscle samples
11 was carried out on approximately 1 cm² blocks cut
12 from the centre of the *longissimus dorsi* muscle.
13 Care was taken to ensure that the same area was
14 sampled from each of the chops. These cubes of
15 muscle were orientated for transverse sectioning,
16 mounted on a piece of cork with optimal cutting
17 temperature compound (OCT), covered with more OCT
18 and with unperfumed talcum powder and frozen in
19 liquid nitrogen with constant agitation. Twelve
20 blocks were taken from each chop and once frozen,
21 were stored in aluminium tins submerged in liquid
22 nitrogen. Throughout the period of the study the
23 blocks were maintained in the liquid phase of the
24 nitrogen dewar to limit any freeze drying. The tins
25 were removed from the liquid nitrogen storage and
26 placed in the cryostat at -20°C 2 hours before
27 sectioning. Serial transverse sections were cut at
28 10µm using a Frigocut 2800 cryostat with motor
29 driven cutting stroke to reduce variation in
30 section thickness.

1 The sections were allowed to air dry at ambient
2 temperature for 2 hours and then frozen overnight
3 for staining the following day.

4
5 The characterisation of fibre typing adopted in
6 this study is based upon the reaction of individual
7 fibres to a minimum of three stains. The stains
8 used were chosen to demonstrate the activities of
9 Ca^{2+} activated myofibrillar adenosine triphosphatase
10 (ATPase), nicotinamide adenine dinucleotide
11 diaphorase (NADH), and α -glycerophosphate
12 dehydrogenase (GPOX), which then allowed the
13 characterisation of the fibres based on their
14 contractile and metabolic activities as follows and
15 as illustrated in Table 2; ATPase - contractile
16 activity (fast or slow twitch); NADH - oxidative
17 activity; GPOX - glycolytic activity.

18

1 Table 2 The histochemical basis of
2 characterisation of muscle fibre types in pig meat.

FIBRE TYPE	STAIN		
	ATPASE	NADH	GPOX
FOG	++(+)	+++	+++
FG	+++	+	+++
SO	+	+++	+

3
4 Quantification of fibre type and size
5
6 Quantitative assessments of fibre type and size
7 were made from the stained muscle preparations
8 using a Torch computer based image analysis system
9 (Vision Dynamics, Hemel Hempstead, Herts).
10 Measurements of fibre size were made on the
11 sections reacted to demonstrate the activity of
12 ATPase. For each animal, fibre size estimation was
13 carried out on eight blocks with two fields per
14 block being analysed.
15
16 The ATPase stained sections were examined under a
17 light microscope fitted with a Sony video camera,
18 the output of which was applied to the image
19 handling software of the Torch computer. The use of
20 the ATPase stain generates an image in which three
21 fibre types can be distinguished based on their
22 grey levels. Fibre type was confirmed through
23 examination of printed images of the NADH and GPOX

1 stains to give information on the metabolic
2 character of each fibre. The three fibre types were
3 analysed separately, and thresholding was altered
4 to detect all fibres of the same type. Where
5 adjacent fibres were thresholded and detected as a
6 single unit, manual editing operations were
7 undertaken to separate the fibres through the use
8 of a superimposed 'live' camera image to visualise
9 the sarcolemmal membranes accurately. The data for
10 size, frequency and percentage area was computed
11 for each animal. Approximately 1600 fibres were
12 analysed for each pig.

13

14 DNA samples were shipped to GeneSeek Inc (Lincoln,
15 Nebraska USA) for genotyping. Marker alleles were
16 amplified by PCR and scored following
17 electrophoresis using infrared fluorescent
18 technology. Markers were amplified using either 1)
19 end-labelled forward primers, or 2) M13-tailed
20 forward primers. Labelled forward primers were
21 synthesised by LI-COR (Lincoln, Nebraska USA),
22 while M13-tailed forward primers and all reverse
23 primers were synthesised by Research Genetics
24 (Huntsville, Alabama USA).

25

26 End-labeled reactions used 25 ng genomic DNA, 200 μ M
27 each dNTP, 0.15 picomol of labeled forward primer
28 (either IR700 or IR800; LI-COR), 1 picomol of
29 unlabeled reverse primer, 0.5 U Taq-Gold polymerase
30 with supplied $MgCl_2$ -free buffer (Perkin-Elmer;
31 Foster City, California USA), and 2.5 mM $MgCl_2$.
32 M13-tailed reactions were the same except that 0.3

1 picomol of each primer were used. Each forward
2 primer had a 19-bp 5' tail consisting of M13
3 sequence, and each PCR included 0.3 picomol of a
4 fluorescently labelled 19-bp M13 primer (either
5 IR700 or IR800). Amplification began with an
6 initial denaturation at 95°C for 5 minutes, followed
7 by "touchdown" PCR with annealing temperatures
8 beginning at 68°C and decreasing by 2°C per cycle
9 through to 54°C. A total of 33 cycles was performed
10 at an annealing temperature of 54°C. PCR ended with
11 a 7 minutes extension period at 72°C. PCR products
12 were denatured at 95°C prior to electrophoresis
13 (1500V, 50mA,
14 50W, 45°C) in 7.0% denaturing polyacrylamide gels in
15 LI-COR (Model 4200 IR2) sequencers.

16
17 Alleles were scored based on size relative to known
18 DNA size standards. Genotyping results were stored
19 in Excel files and delivered to the Roslin
20 Institute as e-mail attachments and loaded into the
21 resSpecies database (<http://www.resSpecies.org>) at
22 Roslin.

23
24 Details of the pedigree structure, dates of birth,
25 sex and growth rate, carcass and slaughter
26 characteristics, sensory and shear force
27 evaluations and muscle fibre characteristics were
28 loaded into the resSpecies database
29 (<http://www.resSpecies.org>) at Roslin Institute
30 from Excel spreadsheets provided by the Rowett
31 Research Institute.

1

2 The collated data on traits and marker genotypes
3 were analysed to scan the genome for the presence
4 of QTL influencing the traits of interest.

5 The animals were genotyped for the genetic markers
6 listed in Table 3. The markers were chosen to
7 provide a reasonable spread over the whole of the
8 genome.

9

1 Table 3: Markers used for genome scan.

Marker	Chromosome	Position
SW1515	1	16
SW1417	1	44
SW1430	1	59
S0331	1	73
SW974	1	103
SW2512	1	144
SWC9	2	1
SW575	2	32
SW1026	2	61
SWR2157	2	89
S0036	2	132
SW2429	3	17
S0206	3	42
SW902	3	58
SW142	3	81
SW349	3	113
SW2404	4	0
S0301	4	27
S0175	4	56
SW512	4	81
SW445	4	106
SW1461	4	120
SW413	5	9
SW1482	5	39
SW439	5	72
S0005	5	88
SW904	5	107

SWR1112	5	130
SW2535	6	18
SW1038	6	47
DG87	6	63
SW709	6	89
S0121	6	116
DG93	6	122
SW2419	6	161
S0025	7	4
SW2155	7	33
TNFB	7	58
SWR1928	7	79
SW252	7	99
S0101	7	135
SW764	7	156
S0353	8	12
SWR1101	8	38
S0086	8	62
SW2160	8	80
SW790	8	108
S0178	8	128
SWR68	9	4
S0024	9	27
SW827	9	54
SW727	9	77
SW539	9	79
SW2093	9	103
SW2116	9	130
SWR136	10	7
SW497	10	39

SWR198	10	65
SW1991	10	80
SW1626	10	104
SW2067	10	124
SW1632	11	17
S0071	11	50
SW435	11	59
SW13	11	86
S0229	12	20
SW1307	12	40
SW874	12	65
S0090	12	80
SW2180	12	105
SWR1941	13	14
SW344	13	36
S0068	13	62
SW1386	13	77
SW1056	13	96
SW2097	13	121
SW857	14	8
SW1027	14	22
SWR84	14	52
SW761	14	76
SWC27	14	112
SW1416	15	13
chr1-4	15	29
SW964	15	51
SW1683	15	79
SW1983	15	102
SWR312	15	120

38

SW813	16	6
SW2411	16	17
SW81	16	40
SW2517	16	56
S0105	16	93
SW335	17	0
S0296	17	32
S0359	17	68
S0332	17	89
SW1023	18	5
SW1984	18	30
S0177	18	55
SW949	X	0
SW980	X	12
SW2126	X	35
SW1943	X	87
SW1608	X	102
SW2588	X	128

1
2 Linkage maps of each pig chromosome were developed
3 using Cri-Map version 2.4 (Green et al 1990). The
4 linkage map positions for the markers on
5 chromosomes 5 and 9 are presented in Table 3. The
6 trait data and linkage maps were analysed by the
7 least squares approach as described by Haley et al,
8 1994. All chromosomes were tested in this way
9 (using appropriate markers for the chromosome under
10 test), but the most significant correlation was
11 found for meat quality with the markers on
12 chromosomes 5 and 9.

13

1 Other more minor effects are given below in Table
2 4.

3

4 Table 4:

5

Chromosome	Trait
3	Total area (FG + FOG)
7	First force, peak force, total work, SO count, SO/cluster

6

7 Analyses

8

9 All QTL analyses were performed by least squares.
10 The assumption underlying these analyses is that
11 QTL of major (i.e. detectable) effects were fixed
12 for alternative alleles in the Duroc and Large
13 White breeds that went into the study.

14

15 The models included fixed effects and any key
16 covariates. Sex was always included as was either
17 year or slaughter data as a fixed effect.

18

19 Results

20

21 The significant results for chromosomes 5 and 9 are
22 set out in Table 5.

23

Table 5. Genome scan results by chromosome

Trait	Chrom.	Position	F ratio	% var 1	% var 2	Trait s.d.	a	s.e.	d	s.e.	Dominance ratio
Clusters	5	0	3.04	3.58	6.9	3.27E-01	-6.19E-02	4.55E-02	1.48E-01	7.45E-02	-2.39
1st force	5	9	5.21	7.49	19.81	5.54E+02	-2.22E+02	8.68E+01	-3.81E+02	1.69E+02	1.72
Peak force	5	9	4.87	6.92	18.28	5.53E+02	-2.20E+02	8.69E+01	-3.56E+02	1.69E+02	1.62
Total work done (shear)	5	14	5.62	8.16	20.34	1.06E+03	-5.25E+02	1.71E+02	-6.01E+02	3.45E+02	1.14
Total area (FG+FOG+SO)	5	30	3.01	3.53	6.98	6.23E+03	1.49E+03	9.40E+02	-2.52E+03	1.62E+03	-1.69
FG/FOG	5	63	4.85	6.54	13.33	8.56E+03	3.50E+03	1.26E+03	3.81E+03	2.16E+03	1.09
FG/FOG %	5	65	6.48	9.06	15.84	2.20E+00	1.14E+00	3.25E-01	6.89E-01	5.79E-01	0.60
SO %	5	65	6.48	9.06	15.84	2.20E+00	-1.14E+00	3.25E-01	-6.89E-01	5.79E-01	0.60
SO area	5	68	6.17	8.6	14.69	5.40E+03	-2.78E+03	8.07E+02	-1.29E+03	1.50E+03	0.46
Boar flavour (Adj.)	5	69	4.69	6.29	15.59	6.48E-01	-2.06E-01	9.78E-02	-4.20E-01	1.83E-01	2.04
PH 45 minutes	5	79	7.1	9.99	14.4	2.26E+01	-5.14E+00	2.92E+00	-1.55E+01	4.63E+00	3.02
Overall acceptability (Adj.)	5	98	3.49	4.33	9.78	1.80E+00	-6.30E-01	2.69E-01	-6.91E-01	5.01E-01	1.10
Juiciness (adj.)	5	98	4.97	6.73	12.47	1.96E+00	-8.63E-01	2.89E-01	-6.55E-01	5.38E-01	0.76
Pork flavour (Adj.)	5	111	4.34	5.72	17.57	1.37E+00	-6.16E-01	2.34E-01	-7.53E-01	4.83E-01	1.22
Abnormal flavour (Adj.)	5	120	4.09	5.32	18.7	8.76E-01	-1.44E-01	1.43E-01	7.30E-01	2.74E-01	-5.07
Clusters	9	0	4.37	5.78	62.71	3.27E-01	6.47E-03	7.27E-02	-5.18E-01	1.75E-01	-80.06
Hue	9	0	4.19	5.48	49.17	3.13E+00	1.11E+00	6.96E-01	-4.10E+00	1.68E+00	-3.69
Light	9	0	4.58	6.11	63.05	1.90E+00	3.32E-01	4.21E-01	-2.98E+00	1.02E+00	-8.98

Peak force	9	1	3.5	4.58	19.3	5.53E+02	3.42E+02	1.30E+02	4.38E+01	3.02E+02	0.13
1st force	9	2	3.05	3.79	16.65	5.54E+02	3.20E+02	1.30E+02	4.88E+00	3.03E+02	0.02
SO/clust	9	2	9.74	13.71	118.12	8.29E-01	-2.52E-01	1.74E-01	1.77E+00	4.23E-01	-7.02
SO count	9	3	3.66	4.61	39.31	1.97E+00	-7.09E-01	4.30E-01	2.25E+00	1.05E+00	-3.17
Total work done (shear)	9	4	3.52	4.62	18.77	1.06E+03	6.40E+02	2.42E+02	1.48E+02	5.70E+02	0.23
Tenderness (Adj.)	9	13	3.33	4.06	18.8	2.15E+00	-9.56E-01	4.16E-01	-1.28E+00	9.71E-01	1.34
pH 24 hours	9	75	4.23	5.54	19.68	9.53E+00	5.01E+00	1.83E+00	4.61E+00	3.60E+00	0.92
pH 45 minutes	9	75	3.72	4.71	14.07	2.26E+01	1.19E+01	4.37E+00	1.96E+00	8.58E+00	0.16
Pork flavour (Adj.)	9	105	3.91	5.03	14.3	1.37E+00	1.92E-01	2.36E-01	-1.00E+00	3.97E-01	-5.21
FG/FOG %	9	121	7.59	10.7	19.78	2.20E+00	-8.67E-01	3.23E-01	1.52E+00	5.50E-01	-1.75
SO %	9	121	7.59	10.7	19.78	2.20E+00	8.67E-01	3.23E-01	-1.52E+00	5.50E-01	-1.75
SO area	9	121	7.13	10.03	18.68	5.40E+03	2.09E+03	7.97E+02	-3.61E+03	1.36E+03	-1.73
FG/FOG	9	123	4.73	6.35	11.53	8.56E+03	-2.75E+03	1.23E+03	4.32E+03	2.04E+03	-1.57
Lean %	9	126	3.78	4.86	8.68	2.27E+00	2.77E-01	3.08E-01	-1.28E+00	4.84E-01	-4.62

1 Notes to Table 5:

- 2 - position is in relation to the first marker, add the
3 position of the first marker for equivalence to USDA
4 maps.
- 5 - %var1 = variance explained (reduction in residual)
6 when QTL (a and d) are included in the model.
- 7 - %var2 = variance predicted from estimated a and d
8 effects.
- 9 - a = additive effect Du-LW, positive means a higher
10 value in Du.
- 11 - d = dominance effect, positive indicates a higher
12 value, heterozygote is above the mean of the two
13 homozygotes.

14

15 The results of the analysis for chromosome 5 are
16 summarised in Figure 2 for muscle fibre
17 characteristics, tenderness and shear force. It shows
18 that F values peak on chromosome 5 at positions 0 to 50
19 for shear force and around 70 for SO % and SO area. The
20 estimates in Table 5 indicate that lower shear force
21 values and lower SO % and area are associated with
22 Duroc genes.

23

24 The results in Figure 3, show high F values at the
25 bottom of chromosome 9, for SO area and SO%, as well as
26 FG/FOG area. As shown in Table 5, Duroc genes are
27 associated with higher SO area and SO%, but lower
28 FG/FOG area. Not shown in Table 5 is that lower shear
29 forces are associated with Duroc genes in this region.
30 At the top of chromosome 9, high F values are found for
31 SO/cluster as well as peaks for shear force traits,
32 indicating that in this case low SO/cluster and high

1 shear force are associated with 'Duroc' genes (Table
2 5).

4 Example 2

5 QTL analysis - additional animals

6
7 Following the initial whole genome scan described in
8 Example 1 above, further animals recorded for the meat
9 quality traits were genotyped by GeneSeek as described
10 above for genetic markers on chromosome 5 and 9. The
11 trait recording, genotyping and data analyses were
12 carried out as described in Example 1. The results
13 from the analysis of chromosome 5 and 9 for all the
14 trait recorded animals - those described in Example 1
15 plus the additional 62 animals, i.e. a total of 180 -
16 are shown in Table 6.

17
18 Linkage analyses for chromosomes 5 and 9 are shown in
19 the table below in which the published USDA map
20 distances are compared from analysis of Phase 1 and
21 Phase 2 data.

23 Chromosome 5				Chromosome 9			
24 Marker	Consensus	Phase 1	Phase 2	Marker	Consensus	Phase 1	Phase 2
25 SW413	0.0	0.0	0.0	SWR68	0.0	0.0	0.0
26 SW1482	32.0	24.4	24.6	S0024	23.0	15.5	36.4
27 SW439	66.0	62.8	65.5	SW827	49.0	46.9	79.3
28 S0005	82.0	79.9	83.2	SW727	72.0	77.0	- ¹
29 SW904	103.0	90.5	103.7	SW539	75.0	77.6	- ¹
30 SWR1112	124.0	112.3	- ¹	SW2093	100.0	97.8	125.9
31				SW2116	126.0	129.6	155.1

32 ¹: Not included in phase 2

1 The results of the analysis for chromosome 5 are
2 summarised in Figure 4 for muscle fibre
3 characteristics, tenderness and shear force (total work
4 done). It shows that F values peak on chromosome 5 at
5 positions 0 to 50 for shear force (total work done) and
6 around 70 for SO % and SO area. The estimates in Table
7 6 indicate that lower shear force (total work done)
8 values and lower SO % and area are associated with
9 Duroc genes.

10

11 The results in Figure 5, show high F values at the
12 bottom of chromosome 9, for SO area and SO%. As shown
13 in Table 6, Duroc genes are associated with higher SO
14 area and SO%. Not shown in Table 5 is that lower shear
15 forces (total work done) are associated with Duroc
16 genes in this region. At the top of chromosome 9, high
17 F values are found for SO/cluster as well as peaks for
18 shear force traits, indicating that in this case low
19 SO/cluster and high shear force (total work done) are
20 associated with 'Duroc' genes (Table 6).

21

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Table 6. Genome scan results by chromosome for the extended number of animals

Trait	Chrom.	Position	F ratio	% var 1	% var 2	Trait s.d.	a	s.e.	d	s.e.	Dominance ratio
Sos/cluster	5	0	2.83	1.94	3.9609	0.84	0.222	0.101	-0.115	0.159	-0.52
Total work done	5	15	4.83	4.89	13.631	1132.7	-464.5	166	-517.7	311.3	1.11
Mean SO area	5	65	5.09	4.23	8.1271	5878.6	-2360.1	740.4	-306.8	1144.5	0.13
%SO	5	65	5.19	4.53	8.3419	2.38	-0.963	0.3	-0.188	0.463	0.20
SOs/cluster	9	59	7.28	6.36	69.862	0.849	-0.173	0.152	1.398	0.389	-8.08
Total work done	9	78	4.39	4.36	28.237	1132.7	309.7	183.9	1121.3	430.8	3.62
Mean SO area	9	154	6.92	6.02	8.8864	5878.6	1767.4	619.9	-2456.9	994.2	-1.39
%SO	9	155	7.34	6.41	8.8266	2.38	0.727	0.244	-0.971	0.384	-1.34

- 1 Notes to Table 6:
- 2 - position is in relation to the first marker, add
- 3 the position of the first marker for equivalence
- 4 to USDA maps.
- 5 - %var1 = variance explained (reduction in
- 6 residual) when QTL (a and d) are included in the
- 7 model.
- 8 - %var2 = variance predicted from estimated a and
- 9 d effects.
- 10 - a = additive effect Du-LW, positive means a
- 11 higher value in Du.
- 12 - d = dominance effect, positive indicates a
- 13 higher value, heterozygote is above the mean of
- 14 the two homozygotes.

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1 CLAIMS

2

3 1. An assay to identify pigs with a genetic
4 predisposition for improved meat quality,
5 wherein said assay comprises:

6 a) obtaining a DNA sample from a test pig;
7 b) analysing the sample to determine the
8 allelic variant(s) present at at least one
9 genetic marker, wherein said marker is
10 selected from:

11 i) SW413, SW1482, SW439, S0005, SW904 or
12 regions of chromosome 5 spanning
13 therebetween; or

14 ii) SWR68, S0024, SW827, SW727, SW539, or
15 regions of chromosome 9 spanning
16 therebetween; or

17 iii) SW2093, SW2116 or regions of
18 chromosome 9 spanning therebetween;
19 and

20 c) using the genotypic data from said marker(s) to
21 select for pigs of the preferred genotype.

22

23 2. The assay of Claim 1, wherein in step c) pigs
24 with high meat quality traits are selected.

25

26 3. The assay as claimed in either one of Claims 1
27 and 2 wherein said method comprises:

28 a) obtaining a DNA sample from said pig;

29 b) assaying said DNA sample for a sequence
30 identical with or complementary to the genetic
31 markers.

32

- 1 4. The assay as claimed in any one of Claims 1 to
2 3 wherein the sample is analysed to determine
3 the allelic variant(s) present at a genetic
4 marker which is located:
5 i) on chromosome 5 in respect of shear force;
6 ii) between SW1482 and SW904 on chromosome 5
7 in respect of fitness traits; and/or
8 iii) between SWR68 and SW2093 on chromosome 9;
9 and/or
10 iv) between SW2093 and SW2116 on chromosome 9;
11
12 5. The assay as claimed in any one of Claims 1 to
13 4 wherein the sample is analysed to determine
14 allelic variant(s) present at a genetic marker
15 on chromosome 5 and at a genetic marker on
16 chromosome 9.
17
18 6. The assay as claimed in any one of Claims 1 to
19 5 wherein allelic variant(s) present at three
20 or more distinct genetic loci are analysed.
21
22 7. The assay as claimed in any one of Claims 1 to
23 6 which said genetic markers are selected from
24 SW413, SW1482, SW439, S0005, SW904 or regions
25 of chromosome 5 spanning therebetween.
26
27 8. The assay as claimed in any one of Claims 1 to
28 6 which said genetic markers are selected from
29 SWR68, S0024, SW827, SW727, SW539 or regions of
30 chromosome 9 spanning therebetween.
31

- 1 9. The assay as claimed in any one of Claims 1 to
2 6 which said genetic markers are selected from
3 SW2093, SW2116 or regions of chromosome 9
4 spanning therebetween.
5
- 6 10. A method to identify pigs with a genetic
7 predisposition for improved meat quality,
8 wherein said method comprises:
9 a) obtaining DNA samples from a population of
10 pigs;
11 b) genotyping at least a sample of said
12 population for pre-determined markers that
13 map within or close to the QTL for meat
14 quality traits on chromosome 5 and 9 at a
15 location displaying a high F ratio;
16 c) measuring meat quality traits for at least
17 a sample of said population;
18 d) correlating the presence of allelic
19 variants of said markers with said meat
20 quality traits;
21 e) obtaining a DNA sample from a test pig;
22 f) analysing the sample to determine the
23 allelic variant(s) present at a said
24 selected genetic marker; and
25 g) using said marker results to select for
26 pigs of the preferred genotype.
27
- 28 11. The method of Claim 10, wherein said markers
29 are derived from SW413, SW1482, SW439, S0005,
30 SW904, SWR68, S0024, SW827, SW727, SW539,
31 SW2093 or SW2116.
32

- 1 12. The method of Claim 10, wherein said markers
2 which map within the QTL for the meat quality
3 traits of tenderness, shear force or muscle
4 fibre traits.
5
- 6 13. The method of Claim 10, wherein said markers
7 are located between SW1482 and SW904 on
8 chromosome 5, or between SWR68 and SW2093 on
9 chromosome 9, or between SW2093 and SW2116 on
10 chromosome 9.
11
- 12 14. The method as claimed in any one of Claims 10
13 to 13, wherein genotypic data from more than
14 one marker is analysed, and each marker allows
15 the allelic variation at different QTL
16 associated with separate meat quality traits to
17 be predicted.
18
- 19 15. The method as claimed in Claim 14, wherein
20 genotypic data from at least three markers that
21 each allow the allelic variation at different
22 QTL associated with separate meat quality
23 traits to be predicted are used in combination
24 to select for pigs of the preferred genotype.
25
- 26 16. The method of any one of Claims 10 to 15
27 wherein said genetic markers are selected using
28 a method selected from the group consisting of
29 microsatellites; restriction fragment length
30 polymorphisms (RFLPs), single strand
31 conformational polymorphisms (SSCP), double
32 strand conformational polymorphisms, single

- 1 nucleotide polymorphisms (SNPs), AFLP™
2 (amplified fragment length polymorphisms, DNA
3 chips, variable number of tandem repeats
4 (VNTRs, minisatellites), random amplified
5 polymorphic DNA (RAPDs), heteroduplex analyses,
6 and allele-specific oligonucleotides (ASOs).
7
- 8 17. The method of any one of Claims 10 to 16,
9 wherein said sample is selected from the group
10 consisting of blood, semen (sperm), ascites and
11 urine, liver tissue, muscle, skin, hair
12 follicles, ear, tail, fat and testicular
13 tissue.
14
- 15 18. A method of selecting pigs for use in breeding
16 programs, said method comprising obtaining a
17 DNA sample from a test pig and analysing said
18 sample to determine the allelic variant(s)
19 present at a genetic marker selected from:
20 i) SW413, SW1482, SW439, S0005, SW904 or
21 regions of chromosome 5 spanning
22 therebetween; or
23 ii) SWR68, S0024, SW827, SW727, SW539, or
24 regions of chromosome 9 spanning
25 therebetween; or
26 iii) SW2093, SW2116 or regions of chromosome 9
27 spanning therebetween; and
28 using the genotypic data from said marker to
29 select for pigs having the required genotype.
30
- 31 19. A kit to identify a pig having a genetic
32 disposition for high meat quality, said kit

- 1 comprising at least three genetic markers
2 having the ability to identify specific allelic
3 variant(s) at three separate QTL indicative of
4 meat quality.
5
- 6 20. A method of determining the genetic
7 predisposition of a pig to yield meat of
8 improved meat quality, said method comprising
9 detecting genes located between the following
10 pairs of markers:
11 i) SW413 and SW904 on chromosome 5;
12 ii) SWR68 and SW539 on chromosome 9; and
13 iii) SW2093 and SW2116 on chromosome 9;
14 wherein said genes are characterised by having
15 allelic variant(s) which can influence meat
16 quality or its component traits, or which are
17 associated with variation in meat quality or
18 its component traits.
19
- 20 21. The method as claimed in Claim 20 wherein the
21 genes are located between the positions of the
22 genetic markers SW413 and SW904 on chromosome
23 5, and variation in said genes influence meat
24 quality or its component traits.
25
- 26 22. The method as claimed in Claim 20 wherein the
27 genes are located between the positions of the
28 genetic markers SWR68 and SW539 or between
29 SW2093 and SW2116 on chromosome 9, and
30 variation in said genes can influence meat
31 quality or its component traits.
32

- 1 23. The method as claimed in Claim 20 wherein the
2 genes are located between the positions of the
3 genetic markers SW413 and SW904 on chromosome
4 5, and variation in said genes associated with
5 variation in meat quality or its component
6 traits.
7
- 8 24. The method as claimed in Claim 20 wherein the
9 genes are located between the positions of the
10 genetic markers SWR68 and SW539 or between
11 SW2093 and SW2116 on chromosome 9, and
12 variation in said genes are associated with
13 variation in meat quality or its component
14 traits.

1/7

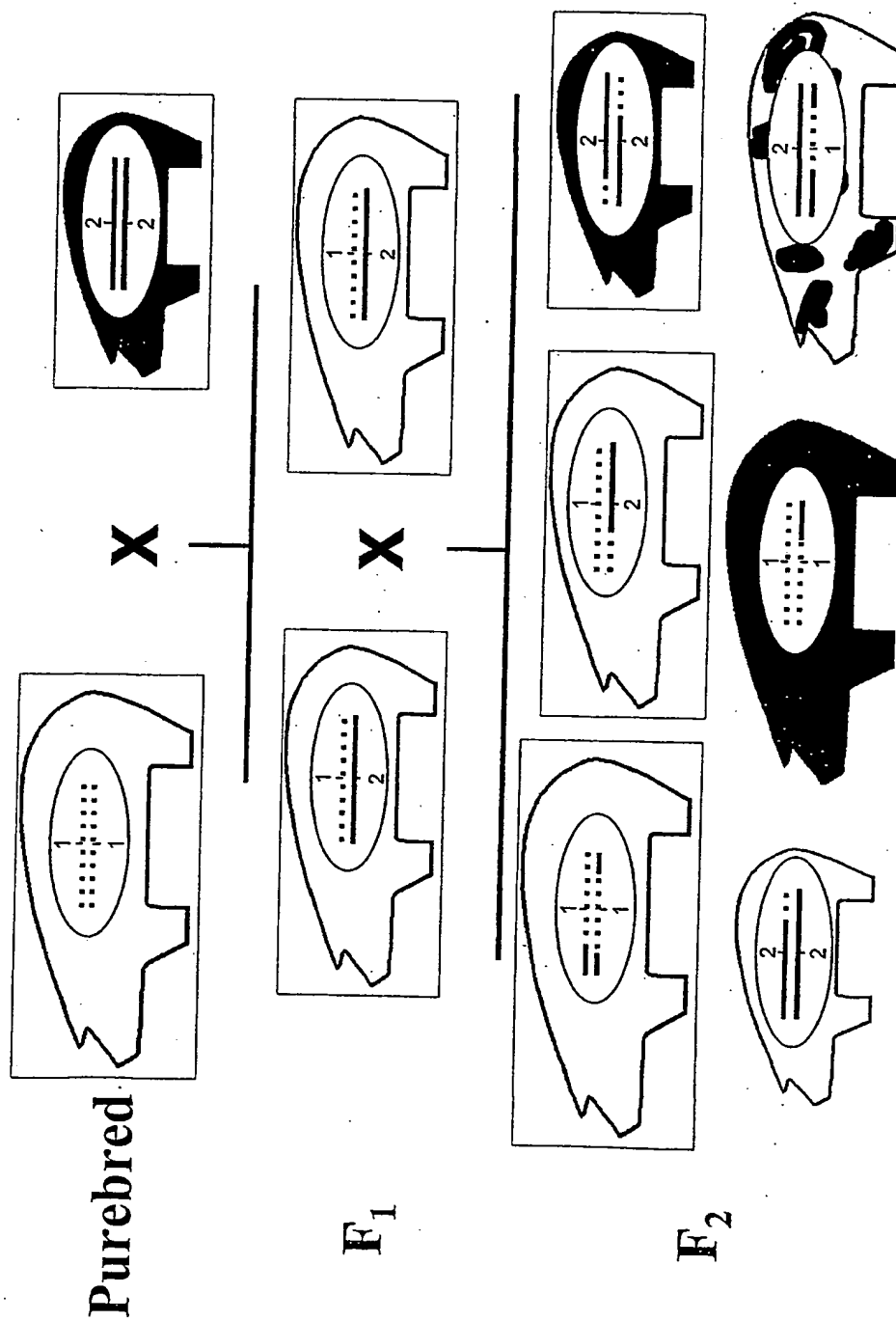


Fig. 1

2/7

Figure 2a. Chromosome 5 QTL scan. Phase 1 after inclusion of inferred genotypes

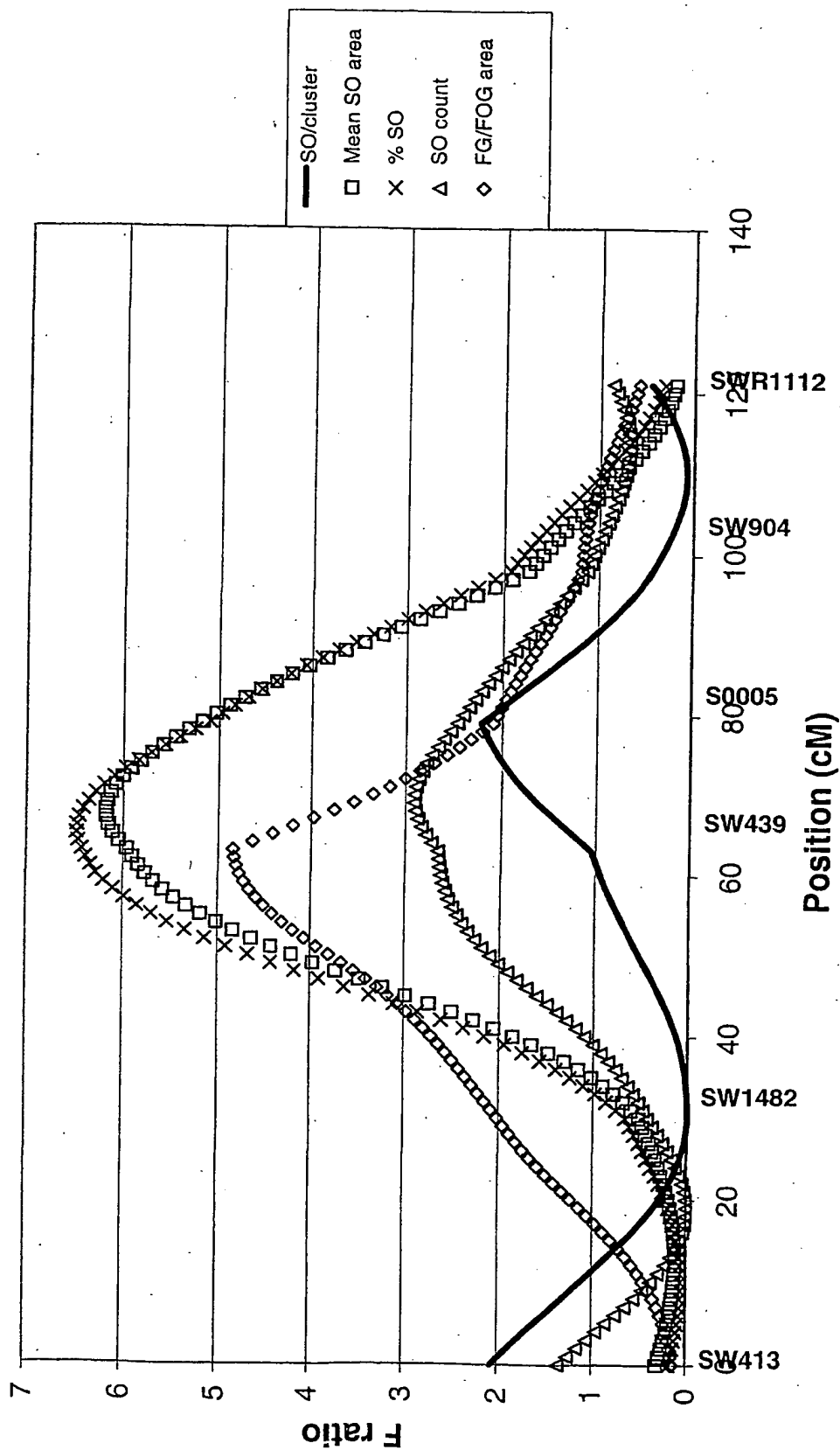


Fig. 2a

3/7

Figure 2b. Chromosome 5 QTL scan. Phase 1 after inclusion of inferred genotypes

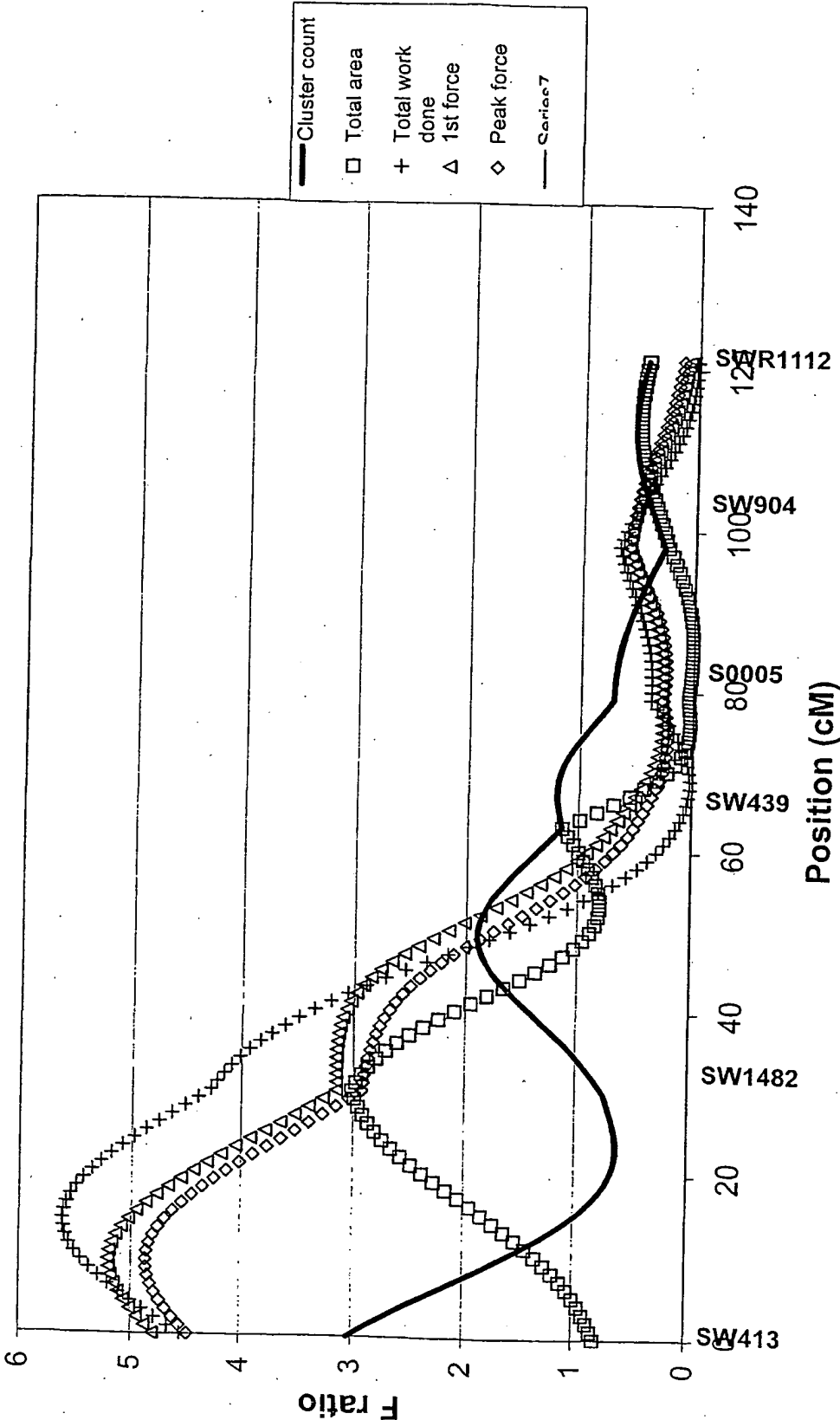


Fig. 2b

4/7

Figure 3a. Chromosome 9 QTL scan. Phase 1 after inclusion of inferred genotypes

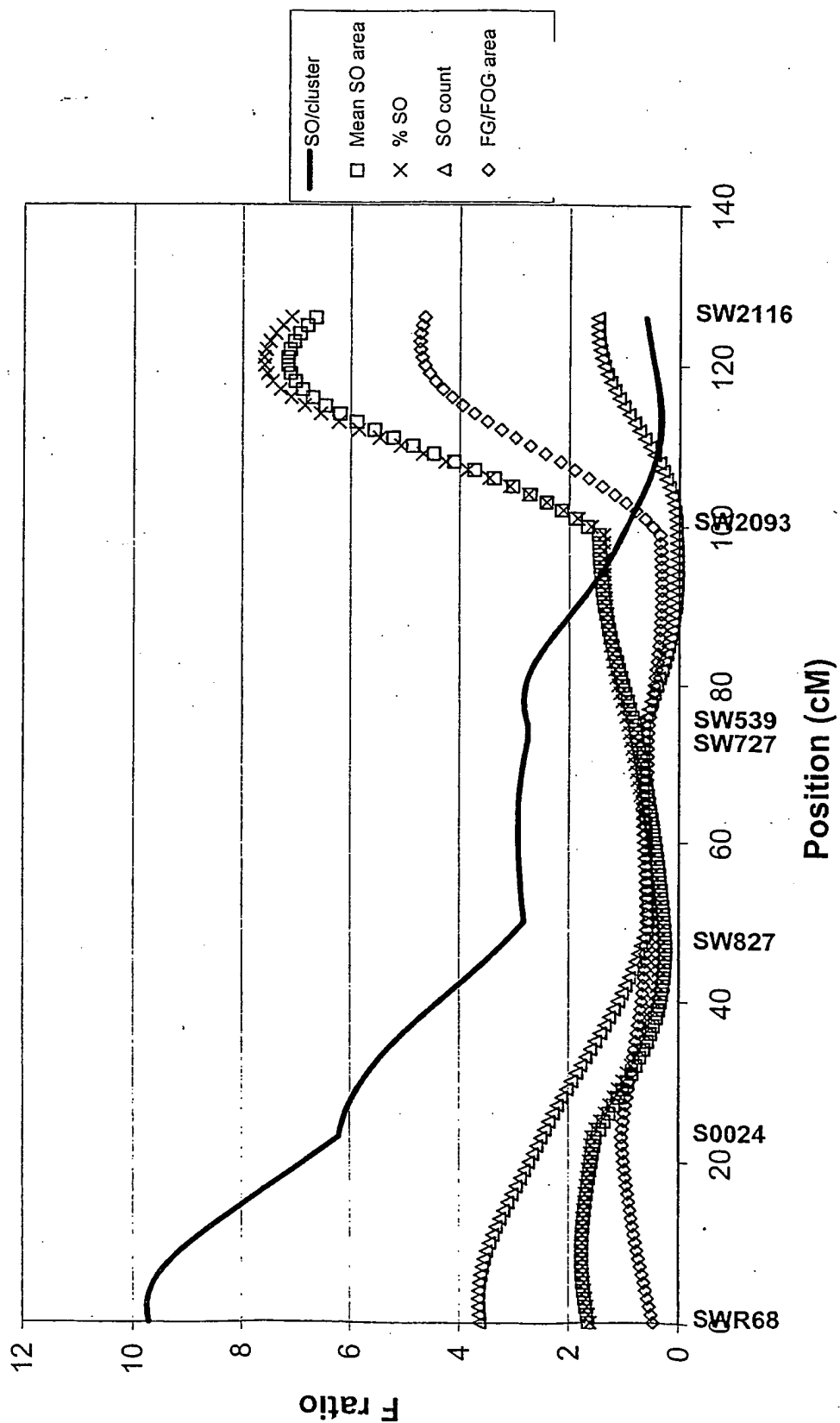


Fig. 3a

5/7

Figure 3b. Chromosome 9 QTL scan. Phase 1 after inclusion of inferred genotypes

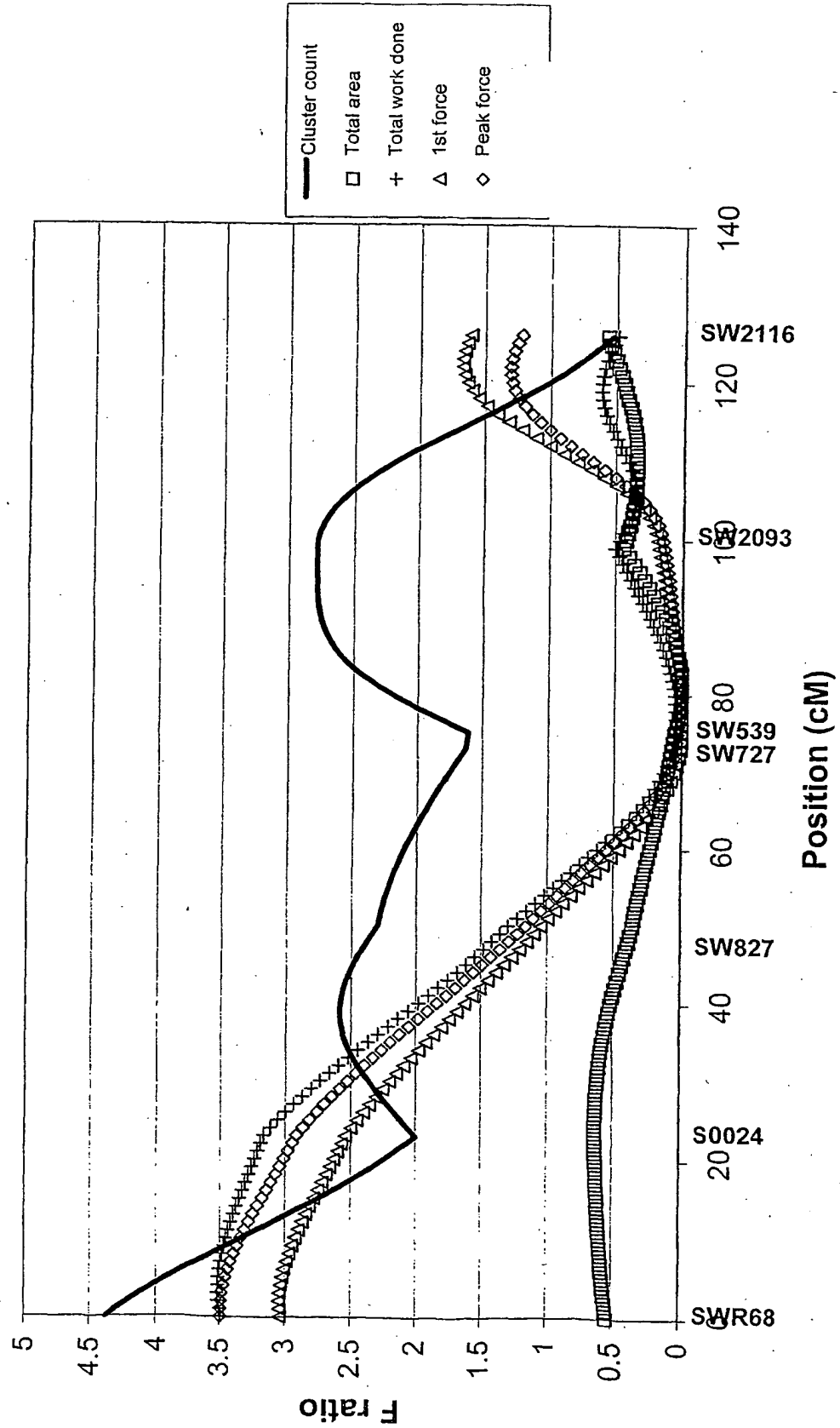


Fig. 3b

6/7

Figure 4. Chromosome 5 Phase 2 results - F2s only

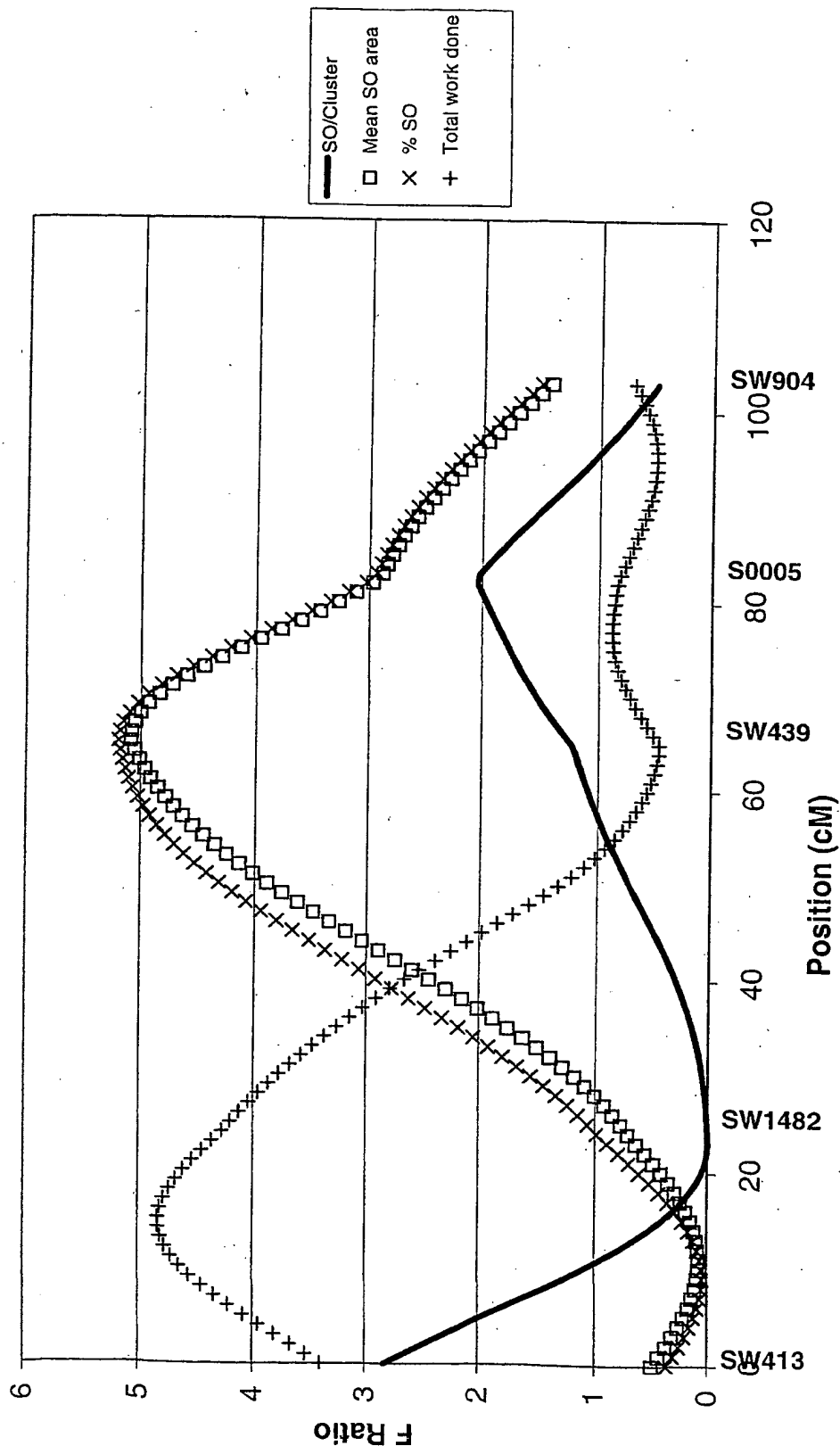


Fig. 4

7/7

Figure 5. Chromosome 9 Phase 2 results - F2s only

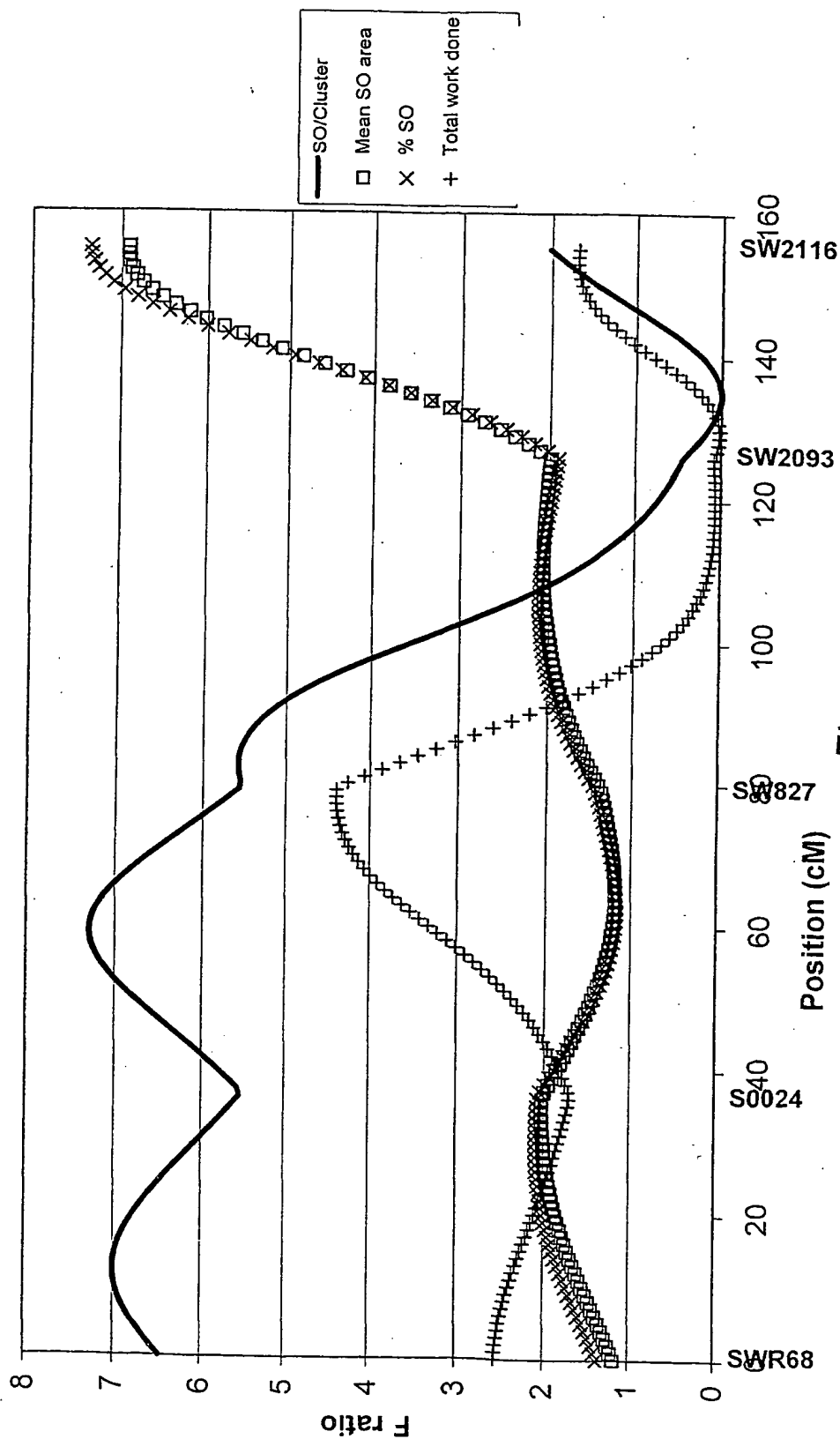


Fig. 5

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